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Water influence on cattle teeth structure

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Abstract. Individual diversity leads to personal taste regarding diet and beverages. Water is the most important nutrient in human survival. Water type and properties directly influences oral cavity's and general health. Teeth's chemical composition is positively either negatively affected by the pH and ions water composition. The objective of this prospective interventional in vitro study was to evaluate chemical enamel and water composition after four weeks of bovine teeth immersion in three different waters: tap, alkaline and mineral. SEM evaluation showed that pH value influences enamel microstructure and microelements dissolution. Teeth storage lead to the readjustment of composition and rheological water properties.

Key Words: water, teeth, enamel, microelements, Scanning Electron Microscope

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Introduction

Geographical, ethnical, social human diversity leads to personal taste regarding one's diet. Beverages are included as part of hydration but as well as a source of taste wellbeing. Most of the people drink tap either bottled water in order to accomplish the daily liquid input. Literature recommends natural mineral water consume as an alternative to hydration, due to the fact that it respects all the hygiene and food security standards; its origin is 100% natural, and its purity leads to no need in disinfection before consume, it has a constant mineral substance composition (which gives to each type of water a specific taste and indications), clinically a high import of micronutrients, if the consume is a regular one (Maraver 2009). Martinez and co. evidenced a number of mineral water characteristics, which make it to improve the health status, especially for aged persons; mineral calcium ions have the same effects as the one diet-sourced, and in absorbs rapidly compared to milk calcium (here's the utility of mineral water in bone degradation prevention); one's micronutrient input must be balanced, to have as less sodium chlorine as it can, so that it won't interfere with high blood pressure either heart failure cardiac diseases; the consume temperature is recommended to be between 12-14 °C, and no more than 12% total carbohydrates (Martinez et al 2008). Researchers from Geothermic Institute (Italy) developed the indications of mineral water based on the literature information (Italiana 2010).

Oral cavity is a dynamic environment, in which dental structures interact with preexistent biofluids (saliva, crevicular fluid), bacteria secreted enzymes. Dental structures are formed by enamel, dentine, cementum and dental pulp. Enamel is the hardest human body structure, and approximately 95% consists of hydroxyapatite crystals, 4% water and 1% organic constituents (amelogenine, enameline, ameloblastine proteins) and lipids (Świetlicka et al 2019). Teeth mechanical properties are directly influenced by the mineralization degree and organic substances concentration, and hydroxyapatite axial disposition (Eimar et al 2012). After eruption, both temporary and definitive teeth undergo dento-maxillary system functions, vicious habits, the effect of diet and beverages through the oxidative stress and glycation products resulted from metabolization, which affect collagen fiber structure and reduce enamel's and dentine's fracture strenght (Băbțan et al 2019).

Water is the main hydration element, and its quality has a direct effect on oral and general health status (de Sousa et al 2012). Both carbonated and non-carbonated water have a complex chemical composition (metals/semimetals – aluminium, cadmium, iron, magnesium, copper) and can be polluted by chemical residuals and microorganisms (Sousa et al 2011). For this reason, filtration, disinfection and water treatment are indicated, in order to reduce pathogenic bacteria transmission and to reduce health negative chemical elements input (Health 2019). Water influences oral tissue structure, and it has been reported that dehydration affects teeth colour (after more than two hours of dry medium storing (Du et al 2012). Mineralization degree can be affected by low pH water and beverages, carbon dioxide

Table 1. Mineral water classification and indications regarding ions composition

Type of mineral water	Medical indications
Carbonated	Enhances digestion, neutralizes gastric acids
Sulfurous	Moderate laxative, liver and bladder diseases
Chloride	Intestinal balance, liver, bladder ducts, laxative effect
Calcium based	For teenagers, pregnant women, people with lactate intolerance, aged patients, prevents osteoporosis and high blood pressure
Magnesiac	Stimulates digestion
Fluoridated	Strenght teeth structure, prevents decays, in association to osteoporosis treatment
Ironed	Iron deficiency and anemia syndrome
High sodium concentration	High intensity physical activity, to supply microelements lost by sweating
Low sodium concentration	High blood pressure treatment

Image 1. Dental sample dehydration (A) in thermobaropolymerization oven; dehydration parameters (B)

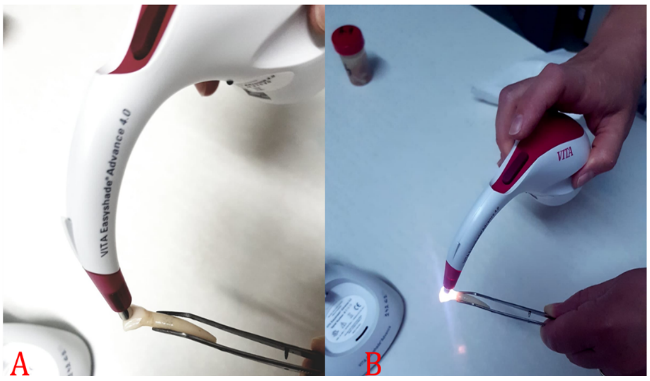

Image 2. Dental colour evaluation using Easy Shade colorimeter, fixed on the incisal third (A) and medium third (B).

Table 2. Immersion waters rheological and chemical characteristics

Parameters	Turbidity (NTU)	pH	Electric conductivity (μS/cm)	Amonium mg/L	Nitrites mg/L	Nitrates mg/L	Calcium mg/L	Magnesium mg/L	Natrium mg/L
Aqua Via	0.72	9.4	280	0.052	<0.008 -0.001	0.514	2.3	0.34	54.2
Perla Moldovei	0.38	8.4	518	0.093	<0.008 -0.001	0.813	2.16	0.3	101.3
Tap water	0.64	7.36	90,7	<0.010	<0.008 (0,001)	2.56	11.2	2.14	2.34

quantity, liquid pigmentations from tea, coffee, red wine, forest fruits (Joiner 2004, 2006). Battersby and co. evidenced that a high hydration enamel degree lead to a low reflection, but sufficient so that the enamel has the same colour with the subjacent dentine (Battersby & Battersby 2015); therefore, enamel has a high implication in colour conversion (for example from yellow-ish dentine to grey enamel). Considering the above presented information, the purpose of the present study was to evaluate dental colour of cattle-derived teeth, before and after immersion in three different types of water, at low temperature (5°C), as well as the influence of teeth structure solubility on water composition.

Material and method

This was a prospective interventional study, in which 6 cattle mandibles were used, 1 to 4 years old aged. Teeth removal was performed in order to harvest 55 samples. Removed teeth were curetted and immersed for disinfection in 0.5% chloramine solution. The samples were introduced in a thermobaromedium (Belle Glass polymerization oven) for 20 minutes at 6 atm and 140°C, for tissue dehydration (Image 1A, B). dental colour was evaluated using a spectrophotometer Vita Easy Shade ® V (VITA Zahnfabrik, H. Rauter GmbH & Co. KG) (Image 2A). for each dental sample, colour evaluation was performed on the vestibular side, in cervical, medium and incisal third (Image 2B). Chemical elements analysis was performed through SEM (Scanning Electron Microscope) assay. After colour evaluation, the teeth were randomly divided in four lots, to be stored at 5°C temperature. The immersion waters (Table 1) were as follows:

- Non-carbonated alkaline mineral water (Aquavia)
- Non-carbonated mineral water (Perla Moldovei)
- Tap water.

For the control samples, distilled water immersion was performed. After the storing during, colorimetric Vita Easy Shade and SEM chemical analysis were performed. Water chemical composition,

Table 3. Rheological and chemical water characterization after 4 weeks of teeth immersion

Parameters	Chlorum mg/L	Fluor mg/L	Sulphite µg/L	Sulphate mg/L	Potasium mg/L	pH	Electrical conductivity (µS/cm)
Aqua Via	3.62	0.3	1	-	0.26	7.84	704
Perla Moldovei	5.96	0.19	0	12.45		7.99	738
Tap water	4.96	<0.10	<5	8.52	0.77	7.23	183

Table 4. ΔE Mean ± DS

Variable ΔE	Mean ± SD Initial	Mean ± SD 2 weeks	Mean ± SD 4 weeks
ΔE	5.3099±3.579	5.801±3.397	5.919±3.585

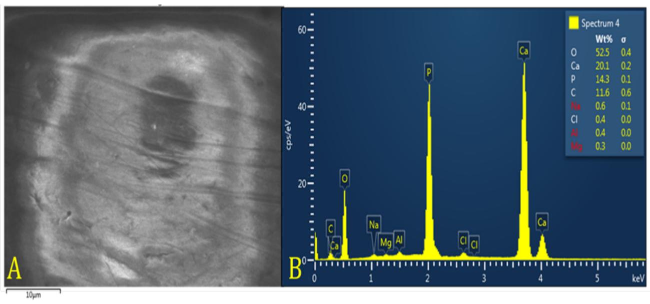

Image 3. Control sample SEM (A), chemical composition (B) before immersion

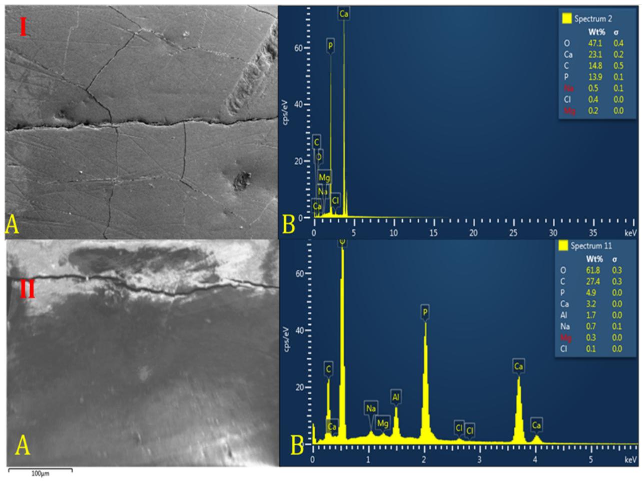

Image 4. SEM micrography (A) before (I) and 4 weeks (II) of mineral water immersion; chemical composition (IB, IIB)

spectophotometric and SEM evaluation were conducted at "Raluca Lipan" Chemistry Institute, Cluj-Napoca.
Statistical analysis was performed using the MedCalc Statistical Software version 19.3.1 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020). Continuous data was expressed as mean and standard deviation. Comparisons between measurements were performed using ANOVA for repeated measures and paired t test. A p value <0.05 was considered statistically significant.

Results

Cattle teeth storage lead to the chemical readjustment of all immersion waters (Table 3). For alkaline water, pH was reduced with 1.56, for non-carbonated water with 0.41 and for tap water with 0.13. Regarding electric conductivity (µS/cm), it increased for all waters, with 124 for alkaline, 220 for mineral non-carbonated and with 92.3 for tap water.
Regarding Easy Shade evaluated colour, ΔE (Table 4) had a linear increase according to immersion time, the highest values being assessed in the first two weeks. Statistical analysis showed no significant differences for ΔE according to immersion water category (p=0.526).
Image 3 exhibits the soft surface (A) of a dental sample from the control lot (immersed in distilled water) before storage; (B) shows high oxygen (O), carbon (C) and phosphor (P) Wt%.
Image 4 shows surface microfractures (A) after thermobarodehydration process; 4 weeks storage lead to significant reduction in canal depth, a positive ions modification regarding O, C, P, natrium (Na), magnesium (Mg) and negative for Cl, Ca (B).
Image 5 evidences surface structure (IA) for tap water immersed sample; 4 weeks storage lead to enamel microsurface negative impact, with depth and area initial microfractures evolution (IB); Chemical analysis (IB, IIB) showed a positive increase for O, C, Na Wt% and negative for Ca, P, Cl and Mg.
Image 6 describes initial (IA) 4 weeks immersion modifications reported to alkaline mineral water immersion. It has been noticed (IIA) a reduction in surface rugosity and the presence of less depth of initial compared to alkaline either tap water immersion. Wt% chemical composition (IB, IIB) was positively modified for O, C, Na and negatively for P, Ca, Cl. SEM identified F ions, which in the initial evaluation were not detectable.
In Table 5 are presented mean and standard deviation (SD) for SEM teeth chemical analysis, initial, at one, two, three, four weeks from the immersion. O had the highest % increase – 3% in the first week, a 1% reduction in the second, and an ascending evolution for weeks 3 and 4. There were no significant differences for dental O Wt% between week 1 and 2 comparing alkaline and non-carbonated mineral water (p=0.136, p=0.133), or between tab water and distilled water (p=0.343, p=0.510). There were no significant dental O Wt% differences between week 3 and 4 comparing tap and distilled water (p=0.757).
Ca Wt % had a 20% reduction in the first 3 weeks, and between weeks 3 and 4 a low Wt% increase compared to the initial value. There were no significant differences for teeth Ca Wt% between week 1 and 2 regarding alkaline, non-carbonated or distilled water (p=0.959, p=0.711 and p=0.336 respectively). There were no differences for Ca Wt% between week 2 and 3 regarding tap water and non-carbonated mineral water (p=0.437, p=0.161).
C Wt % had a 20% reduction in the first 2 weeks, between week 2 and 4 a 10% increase according to the initial value. There were no statistically significant differences of teeth C Wt% between week 1 and 2 for alkaline and non-carbonated mineral water (p=0.818, p=0.389), tap water and distilled water (p=0.715 and p=0.184). Between week 2 and 3 there were no

Table 5. Dental SEM chemical analysis according to immersion duration

Variable	Immersion duration (Mean± DS)				
Chemical element Wt%	**Initially**	**1 week**	**2 weeks**	**3 weeks**	**4 weeks**
O	53.175±12.018	56.500±10.251	55875±10.207	56.750±12.018	57.225±4.785
Ca	15.375±12.192	15.525±5.253	14.475±9.067	12.200±8.015	12.675±8.384
C	20.833±5.723	19.400±4.936	16.775±2.205	18.775±6.483	22.567±6.726
P	10.300±4.953	11.550±4.652	11.025±3.644	10.200±4.246	10.725±4.764
Na	0.650±0.208	0.825±0.263	0.700±0.216	0.750±0.129	0.755±0.125
Cl	0.275±0.206	.0275±0.170	0.275±0.170	0.250±0.129	0.300±0.182
Mg	0.225±0.050	0.225±0.050	0.275±0.095	0.325±0.050	0.275±0.095

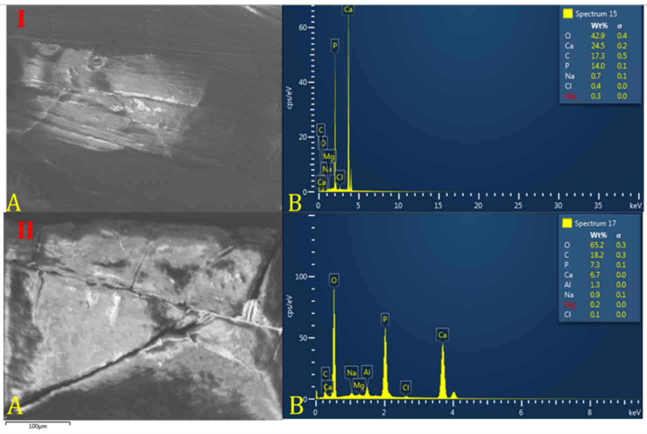

Image 5. SEM micrography (A) before (I) and 4 weeks (II) of tap water immersion; chemical composition (IB, IIB)

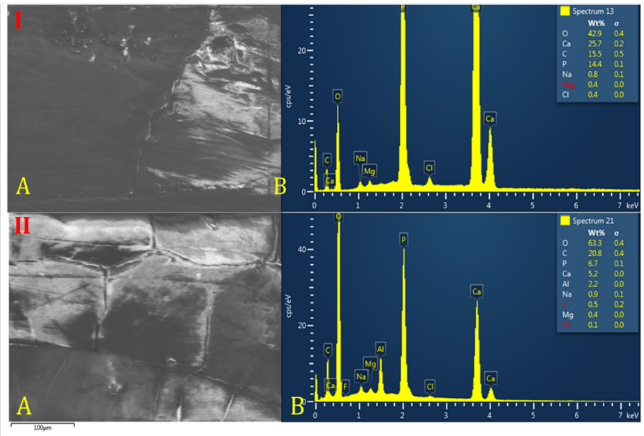

Image 6. SEM micrography (A) before (I) and 4 weeks (II) of alkaline mineral water immersion; chemical composition (IB, IIB)

differences of C Wt% regarding tap and non-carbonated mineral water (p=0.659, p=0.489).

P Wt% had a 10% reduction in the first 2 weeks, and a 5% increase in the last 2 weeks, considering the initial value. There were no significant differences of teeth P Wt% between week 1 and 2 for non-carbonated mineral water and tap-water (p=0.454, p=0.508), or for tap water and distilled water (p=0.837 and p=0.671). Between week 3 and 4, there were no significant differences for tap water and distilled water (p=0.216 and p=0.503).

Na Wt% (Figure 6) had an approximately 20% increase in the first 2 weeks, and for the week 3 and 4 a fluctuating evolution, the final NA Wt% being with 10% higher according to the initial value. Teeth Na Wt% had no significant differences between week 1 and 2 for alkaline and tap water (p=0.069 and p= 0.495), distilled and tap water (p=0.092 and p=0.789). between week 2 and 3 there were no Na Wt% differences for non-carbonated mineral and tap water (p=0.239, p=0.495). between week 3 and 4 there were no identified significant differences between alkaline mineral and distilled water (p=0.342 and p=0.789).

Regarding teeth Cl Wt%, it presented a constant % in the first 2 weeks, between week2 and 3 a 5% reduction and between week 3 and 4 a 5% increase according to the initial value. Statistical analysis showed no significant Cl Wt% differences between week 1 and 2 for alkaline, non-carbonated mineral, tap or distilled water (p=1.000, p=1.000, p=0.638 and p=0.182). Between week 3 and 4 there were no significant differences of Cl Wt% for non-carbonated, tap or distilled immersion water (p=0.391, p=0.182).

Mg Wt% was modified in weeks 1-3, when it had a 0.10 increase, and the last evaluation showed a 0.05 reduction according to the initial value. There were no statistically significant differences of Mg Wt% between week 1 and 2 for alkaline, tap, non-carbonated mineral and distilled water (p=1, p=0.391, p=0.182 și p=0.495). between week 2 and 3 there were no differences of Mg Wt% for the non-carbonated mineral and tap water (p=0.182).

Discussions

Bottled water drinking has increased due to the lack of confidence that people have in tap water, either ground water, by the numerous contained disinfection chemical substances, toxic waste and the fact that is seems easier and more comfortable to consume presecured water (Saylor et al 2011). Governmental authorities recommend daily water input beside hydration purpose, as an exogenous Ca and Mg source (Penn et al 2009). Intraoral pH has a 7.4 mean value (6.8 for non-stimulated and 7.8 for stimulated saliva), which is easily reduced during sleep (Loke et al 2016). Saliva has in its constitution C, F, P ions, which delay enamel degradation process (Ilea et al 2019); HA crystals have Ca, Mg, P, K, hydroxide ions, which react in saliva contact, so that carbonates replace P and hydroxide ions might replace F (Shellis et al 2014). Thus, it has been suggested that dental tissue degradation depends of Ca, F, P and saliva/immersion solution concentration (Lussi et al 2014). Oral tissues have a 1 μm thickness non-stimulated saliva pellicle protection, which might be easily displaced by acid substances, especially due to the fact that saliva has a decreased buffer capacity compared to stimulated saliva (Dawes 2008). The type and buffer capacity if beverages influence the demineralization process. As higher de buffer capacity, as much they can maintain a low oral pH (Loke et al 2016). Mineral water have a 4.5-9.5 pH variation, 2.8-4.9 electrolyte beverages, 6.4-6.8 milk, 2.9-8.0 for chlorinated poll waters (Lussi et al 2004). Also, drugs (C vitamin, aspirins, chemotherapeutics, opioids), diseases (gastro-oesophagian reflux), alcohol, contribute to pH value reduction and severity of dental demineralization (Scheutzel 1996).

Literature studies obtained values of 15.49 mg/L for Cl, 1.83 mg/L for potassium, 7.75 mg/L for sulphates, a 369 µS/cm turbidity and 8 pH for tap water (Mohseni-Bandpei et al 2018), results which are 2 folds higher compared to the ones from the present study, most likely due to the fact that water samples were collected from depth sources. Vannuci's results are in agreement to ours regarding potassium, sulphates but different regarding F, Cl, ions, our results showing 2 folds lower values (Vannucci et al 2018). Parry and co. evaluated non-carbonated and carbonated commercial available waters, where they found a 7.2-8.1 pH, 0.5 mg/L Ca ions, in agreement with the ones presented at Results section, and for carbonated water 5.05 pH and 0.05 mg/L Ca ions (Parry et al 2001). They showed that carbonated mineral water consume has a high enamel demineralization potential, especially in the presence of Mg and sulphate ions. Io our study we noticed an increase in Mg in enamel structures, but there was no difference between the immersion waters. Creteanu analyzed the correlation between mineral water and dentine regarding Ca, F and Mg ions (Razvan et al 2020). Wt% values were higher compared to ours, and dentine and water ions were positively corelated, meanwhile we found no correlation between SEM analysis and the immersion water type. Noy and al evaluated (SEM) enamel postbirth chemical structure, after storage in tap and desalinated water; their results (Wt% C- 12.62, Wt% O – 17.85, Wt% Mg – 0.195, Wt% P – 19.32, Wt % Ca – 49.72) differed from the ones in the present study, most likely due to the tissue's age difference (Noy et al 2020). Cattle teeth are frequently used for in vitro studies, due to the similarities and the possibility to compare them with human teeth. Cakir and al SEM evaluated frontal bovine teeth before and after whitening protocols application (2011). Wt% for Ca and P were higher than ours, very similar for Mg and O, and lower for Na. In our study, SEM microphotographs evidenced the effects of different types of immersion waters on dehydrated dental samples. The lowest microfracture depth was observed in alkaline mineral water immersed teeth, followed by tap water. Probably the water's alkalinity and its high buffer capacity counteracted hydroxile ions elimination from hydroxyapatite matrix, and moreover, contributed to the enamels' remineralization process.

Literature states there is no dependent relation between water pH and electrical conductivity (Sechriest 1960). Our results are according to research studies regarding water conductivity (Królak et al 2018; Rango et al 2012). It was notices that the higher the conductivity at the end of the storage period, the lower the Ca Wt% and increase O, C P in dental tissues. It is known that distilled water has the lowest electrical conductivity (0.05 µS/cm) and that along with the diversity of the ions increases the conductivity as well; the highest molar value was found for ammonium, Na, Ca, hydroxile, associated to an increased temperature (Bešter-Rogač and Dušan 2006). Although storage temperature in our study was low (5°C), the doubled conductivity values might be explained by the ions diversity given by the demineralization process and Ca eliminated from the non-organic matrix.

Mousa evidenced that alkaline water intake increases bone mineral density, protein mass, prevents cardiac diseases and osteoporosis, and waters alkalinity influences blood pH, which is directly correlated to oral health status (Mousa 2016). Qui showed on animal model that low mineral waters reduce bone Mg, Ca concentration, decrease fracture strength and collagen fiber regeneration (Qiu et al 2015). Dental medicine doctors try to aware their patients by the direct effect that beverages have on dental structure and strenght, in the occurrence and progression of decays and enamel erosion. Wright et al tested 14 types of bottled water and compared pH given by the producer and the ones they obtained (Wright 2015). They evidenced that 10 of 14 beverages had a lower pH value compared to the official ones. Moreover, adding Water Enhancers substances, in order to modify/remove water taste, leads to liquid pH decrease (Ngoc et al 2018). Critical pH is represented by the highest values at which there is an equal ion change between a solid sample immersed in its saturated solution; it has been demonstrated that enamel erosion has an etiological pH of 5.2-5.5 value, and root dentine erosion 6.8 pH (Fisher et al 2017). Non-carbonated mineral waters have an insufficient ionic concentration for the remineralization process, but they less harmful compared to carbonated waters (Zandim et al 2011), which was positively agreed from our SEM dental images.

Dental color is subjective and differs in humidified and dehydration conditions, and it can be influenced by the beverage type and characteristics. The most frequent for color assessment is by using a standard color shade, but this also depends by illumination conditions, examiners' visual acuity, age and experience (Watts & Addy 2001). A more precise alternative is using a colorimeter which has three parameters: L (luminosity), a (red and green shades), b (yellow and blue shades). ΔE evaluates color adjustments by measuring the distance between two points a and b, using a standard equation (Kennedy 1997), being known the fact that all liquids have a $\Delta E > 2$. Regarding ΔE, Babina's evaluation showed an enamel translucence (dehydrated) with values between 3.9-10.9 (Babina et al 2019), with a 0.05 increase after 2 days of tap water immersion, and Vieira-Junior and co. acquired ΔE=5,54-6,56 (before immersion), respectively ΔE=5, after two weeks of alkaline mineral water immersion (pH=7.6) (Farawati et al 2019). Literature results are in accordance with the ones from the present study, but there is lack of information regarding dental tissue storage in mineral water. There were no significant color differences between the four types of used waters, but ΔE had a directly proportioned value with the storage period.

Conclusions

The type and waters' temperature directly influenced the teeth' mineralization degree. Degradation in the four types of water lead to chemical composition readjustment of the dental structures, and the highest demineralization was found for carbonated mineral water. Surface microstructure was influenced by the contact time with alkaline and carbonated mineral water. It's important to have an efficient doctor-patient communication and awareness regarding low pH beverages intake. Dental medicine doctors should recommend alkaline either neutral pH water in order to prevent dental structure demineralization.

Acknowledgements

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